Comparative Growth of the Ruminal Bacteria *Fibrobacter succinogenes* and *Ruminococcus flavefaciens* on Cellulose and Cellobiose: Analyzing the Cost and Benefit of a Cellulolytic Mode of Growth

P.J. Weimer

Introduction

Cellulose is the primary component of plant cell walls and thus serves as a major feed component in forage-based dairy rations. In the rumen, a specialized group of bacteria converts cellulose to acetic acid and succinic acid (a precursor to propionic acid) used by ruminants for energy and milk production. Growth of the bacteria during the fermentation also results in conversion of ammonia to microbial cell protein, which serves as a protein source for the ruminant. While quantitative information of growth of ruminal cellulolytic bacteria is important for modeling the ruminal fermentation, there is little information available on how microbial growth yield may differ when these bacteria are growing on cellulose versus on soluble sugars that can also serve as growth substrates. Growth on cellulose may require additional expenditures of cellular energy and materials to synthesize cellulolytic enzymes and a glycocalyx that facilitates attachment of cells to cellulose. On the other hand, growth on cellulose can provide energetic advantage through more efficient activation of hydrolytic products via the intracellular enzyme cellodextrin phosphorylase (van Walsum and Lynd 1997). The purpose of this study was to compare the growth yields of two prominent ruminal cellulolytic bacteria on cellulose and cellobiose. By comparing the true catabolic growth yields and maintenance coefficients obtained during growth in media differing only in energy source (cellulose vs. cellobiose), the relative cost or benefit of a cellulolytic mode of growth can be determined.

Materials and Methods

Pure cultures of *Fibrobacter succinogenes* S85 and *Ruminococcus flavefaciens* FD-1 were grown in cellobiose-limited continuous culture under a CO_2 gas phase. The bioreactors (working volume 139 mL or 150 mL) contained a modified Dehority medium supplemented with 25 mL of clarified ruminal fluid and 4 g of cellobiose per liter. Experiments were conducted at seven different dilution rates (range 0.016 to 0.101 h^{-1}). Cultures were sampled 4 to 7 times over a 3 d period after reaching steady state ($\geq$ 4 dilutions). Culture samples (5 mL) were analyzed for residual sugars via a phenol-sulfuric acid assay, for soluble fermentation products (acetate, succinate and formate) by HPLC, and for total particulate N (a measure of cells and cell-associated enzymes and glycocalyx) by combustion analysis. The data obtained from these cellobiose-limited cultures was compared to data obtained for growth on the same medium with cellulose instead of cellobiose (Shi and Weimer 1992, Weimer 1993). Comparisons were made on a N basis to avoid potential confounding by differences in the amounts of intracellular storage polysaccharides.

Results

Growth on cellobiose of both *F. succinogenes* S85 and *R. flavefaciens* FD-1 displayed linear Pirt plots (inverse of observed yield vs. inverse of dilution rate). Comparison of the growth data on cellobiose versus that obtained on cellulose (Table 1) revealed that, for both cultures, the true catabolic yield (Y_G) and the maintenance coefficient (m) were higher on cellobiose than on cellulose.

The parameters Y_G and m were used to solve the Pirt equation at different bacterial growth rates

$$1/Y = 1/Y_G + m/\mu,$$

where Y = observed growth yield and μ = growth rate (equivalent to dilution rate in chemostat culture). The data reveal that these strains display a higher growth yield on cellulose than on cellobiose only at very low growth rates (0.017 h^{-1} for *F. succinogenes* S85, and 0.040 h^{-1} for *R. flavefaciens* FD-1).

Cultures of both strains contained only low levels of extracellular protein, and low activities of extracellular Avicelase and CMCase enzymes. Analysis of fermentation products revealed that the molar yield of

acetate and succinate (the two carbohydrate-derived organic fermentation products of these strains) was considerably lower for cellobiose-grown cultures (Table 1). This value is expected to be 2.0 in the absence of cell growth and anabolism, and in the range of 1.6-1.7 at the growth yields observed in these experiments. It is likely that synthesis and degradation of storage polysaccharides, known to occur extensively in both strains, proceeds to a greater extent during growth on cellobiose, even low at growth rates equivalent to those on cellulose. Calculations based on recovery of carbon in cells, acetate, and succinate reveal that the amount of substrate partitioned into the synthesis and degradation of storage polysaccharides during growth on cellobiose and on cellulose, respectively, were 23 and 13 per cent for *F. succinogenes*, and 27 and 16 per cent for *R. flavefaciens*. Unproductive cycling of substrate through storage polysaccharides may account for the larger maintenance coefficients for these strains on cellobiose.

Conclusions

Under most growth conditions, the cellulolytic mode of growth generally reduces the growth yield of two prominent species of ruminal bacteria. Utilization of cellulose, while less efficient for microbial growth, apparently represents an adaptation to a specialized lifestyle in response to the availability of cellulose, an abundant growth substrate utilizable by few other bacterial species.

References

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Table 1. Growth parameters for *Fibrobacter succinogenes* S85 and *Ruminococcus flavefaciens* FD-1, determined in continuous cultures limited by cellobiose or cellulose.

Strain	Substrate	Y_G (g N/ g AHG)	m (g AHG/ g N/ h)	Mean molar Yield A+S
F. succinogenes S85	Cellobiose	0.041	0.73	1.19
	Cellulose	0.026	0.46	1.35
R. flavefaciens FD-1	Cellobiose	0.085	2.10	1.14
	Cellulose	0.029	1.04	1.45

AHG = anhydroglucose, A = acetate, S = succinate